

# Microbial Pathogens Associated with Proximal Dental Caries in the Primary Dentition and Their Association with Periodontal Disease in Children

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## ABSTRACT

**Background:** Certain specific bacterial species from the subgingival biofilm have demonstrated etiological relevance in the initiation and progression of periodontitis. Among all the bacteria studied, three have shown the highest association with proximal caries and bone loss: *Fusobacterium nucleatum* (Fn), *Capnocytophaga* (Ca), and *Campylobacter rectus* (Cr). Therefore, the relevance of having accurate microbiological diagnostic techniques for their identification and quantification is clearly justified.

**Aim:** To identify the bacterial pathogens with alveolar bone loss and proximal caries in primary dentition and their association with periodontal disease utilizing deoxyribonucleic acid (DNA) microbial probe testing.

**Materials and Methods:** Subgingival plaque samples were collected at baseline as well as at 3, 6, and 12 months later. After extracting DNA, Fn, Ca, Cr, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and several other periodontopathogens were determined by DNA microbial probe testing method.

**Results:** Samples detecting a high bacterial load of Fn, Ca and Cr in children having proximal caries associated with periodontal disease compared to children having proximal caries without periodontal disease ( $P \leq 0.01$ ).

**Conclusions:** Results suggested that there was a relationship between microbial pathogens associated with proximal dental caries in the primary dentition and periodontal disease in children. In addition, DNA microbial probe testing technology clearly analyzed the different loads of periodontopathogens in children who had with proximal caries associated with bone loss and is useful in microbial diagnostics for patients in dental practices.

**Key words:** Periodontal pathogens, periodontal disease in children, proximal dental caries

ملخص البحث :

هدفت هذه الدراسة المستقبلية إلى تحديد مسببات الأمراض البكتيرية المرتبطة بفقدان العظام السنخية في الأسنان الأولية المتسوسة باستخدام اختبارات الحمض النووي للبكتيريا. تم جمع عينات من الترسبات تحت اللثة في بداية البحث وبعد ثلاثة، ستة وأثنى عشر شهراً. بينت الدراسة وجود علاقة بين مسببات الأمراض الميكروبية وتسوس الأسنان الأولية القريبة وأمراض اللثة لدى الأطفال. وقد استخدمت تقنية الـ DNA لتحليل الكثير من مسببات أمراض اللثة لدى الأطفال المصابين بتسوس الأسنان القريبة المرتبطة بفقدان العظم وأثبتت أنها مفيدة في التشخيص الميكروبي في عيادات الأسنان.

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## INTRODUCTION

Gingivitis is a relatively mild gum disease, which without appropriate treatment can lead to periodontitis, a more severe gum disease. Both affect many people, including children, which is when these diseases are usually manifested. In young individuals, gingival and periodontal diseases tend to be less dramatic than in adults.<sup>[1]</sup> The difficulty in diagnosing these diseases is due to the localized nature of these diseases and the less-marked gingival changes, but once detected, they need careful monitoring by the clinician, particularly in children. Clinicians tend to concentrate on the more dramatic and obvious diseases, such as caries, trauma and crowding and can overlook gingival and periodontal diseases.<sup>[2]</sup>

Alveolar bone level in children is measured from the Cemento-enamel junction to the alveolar bone crest (CEJ-ABC). The normal distance between the CEJ and ABC is approximately 2 mm. The presence of any measurement more than 2 mm is indicative of bone loss except in certain situations.<sup>[3]</sup> For example, it can be difficult to diagnose alveolar bone loss in the primary dentition due to increased distance between CEJ and the level of alveolar bone crest.

The distance between the CEJ-ABC is also age dependent. In children, continuous facial growth of the maxilla and the mandible combined with vertical movement of the primary dentition, the distance between the CEJ and ABC increases with age.<sup>[4]</sup> An increase in CEJ-ABC distance that exceeds 2 mm is often in association with primary teeth nearing exfoliation and eruption of the first permanent teeth. This often leads to an angular appearance of the crestal bone that may be mistaken for an angular bone defect.<sup>[5]</sup>

In otherwise normal and stable primary dentition, the CEJ-ABC distance > 2 mm is considered questionable and a distance of 3 mm is considered a definitive pathology. Moreover, an important criterion for the diagnosis of periodontal disease is the loss of lamina dura in association with an increased distance between CEJ and ABC.<sup>[6]</sup> In children, proximal caries in primary molars have been identified as a causative agent for the development of alveolar bone loss. Many investigators have indicated that plaque accumulation and gingival inflammation lead to alveolar bone loss, even though the etiology is still unknown.<sup>[7,8]</sup>

Bimstein *et al.*, studied the relationship between proximal caries and alveolar bone loss in primary dentition.<sup>[7]</sup>

They found that children with extensive proximal decay, defective proximal restorations and stainless steel crowns showed higher prevalence of bone loss. In a later study, Bimstein *et al.*, found no relationship between contact loss and alveolar bone loss, and concluded that bone loss was mainly due to the accumulation of plaque at these sites.<sup>[8]</sup>

In a follow up study, Bimstein *et al.*, attempted to identify the microorganism associated with alveolar bone loss in primary dentition with extensive proximal decay. Although not conclusive, they found higher levels of motile spirochetes and *Actinobacillus actinomycetemcomitans* in sites with alveolar bone loss. The sites that did not show alveolar bone loss displayed a higher count of nonmotile cocci bacteria when compared to sites with alveolar bone loss. In their study, they utilized dark field microscopy and concluded that deoxyribonucleic acid (DNA) microbial testing could be more precise in identifying the microbial pathogens associated with alveolar bone loss in children with proximal decay.<sup>[8]</sup>

Since not all children exhibiting proximal caries or defective restoration exhibit alveolar bone loss, the etiological factor causing the alveolar bone loss is still to be determined. The main causative factor of periodontal disease in humans is known to be bacterial pathogens; five major bacterial species are known to cause periodontitis. The first, *A. actinomycetemcomitans* is a Gram-negative facultative rod known to produce a leukotoxin, which is capable of destroying polymorphonuclear leukocytes (neutrophils). This bacterium also has the ability to invade epithelial cells and connective tissue making it very hard to treat the infected site. The second, *Prophyromonas gingivalis*, is an asaccharolytic, Gram-negative anaerobic rod, which is normally found in the oral cavity with relatively low potency endotoxin, but functions as a significant cytotoxin and a potent inducer of several host-driven cytokines and chemokines. The third, *Prevotella intermedia*, is a saccharolytic, anaerobic, Gram-negative rod, previously classified as *Bacteroids*. It usually occupies oral as well as nonoral sites.<sup>[9]</sup>

The fourth bacterial pathogen, *Bacteroids forsythus*. *B. forsythus*, is a nonmotile, Gram-negative, saccharolytic, anaerobic rod that resembles nonoral *Bacteroids*. These bacteria demonstrate a strong trypsin like benzoyl-DL-arginine-2-naphthylamide (BANA) activity and were known previously as *fusiform Bacteroids*. The fifth bacterial pathogen is *Treponema denticula*, a motile, anaerobic, Gram-negative helical rod. Like *Bacteroids* species, it produces a trypsin like BANA protease.

The primary objective of this study was to identify the bacterial pathogens associated with alveolar bone loss in primary dentition with proximal caries and their association with periodontal disease utilizing DNA microbial probe testing and identify the type of bacterial pathogens associated with alveolar bone loss in primary dentition affected with proximal caries.

## MATERIALS AND METHODS

Twenty-five male children aged between 4 and 6 years were selected to participate in this split-mouth randomized single-blind study conducted at the College of Dentistry, University of Dammam. Participants who had not had a course of antibiotic treatment within the 2 months to the visit and displayed cooperated behavior were considered for inclusion. Patients with sites with alveolar bone loss associated with proximal caries in primary dentition served as the test group ( $n = 25$  sites) [Figure 1] while participants who had sites with proximal caries in primary dentition and no alveolar bone loss served as the control group ( $n = 25$  sites) [Figure 2]. Upon selection, the participants underwent a full oral and radiographic examination to reveal whether the pulpal pathology in the teeth involved in the study was normal.

DNA probing was utilized to analyze the microbial pathogens present in both test and control sites. The distance between the CEJ and the ABC was recorded as a bone loss when it was  $>2$  mm.

Written informed consent was obtained from the patient's parent or guardian prior to start this study and the protocol was reviewed and approved by the Ethical Committee of the College of Dentistry, University of Dammam, Saudi Arabia. Before the children received any treatment, the test and control sites were first isolated using cotton rolls; the gingiva at the test site was dried using  $2 \times 2$  sterile

gauze samples of plaque and crevicular fluid was collected via a paper point and sent for DNA analysis. The children then received regular care (filling the carious teeth) and may participate in follow-up studies.

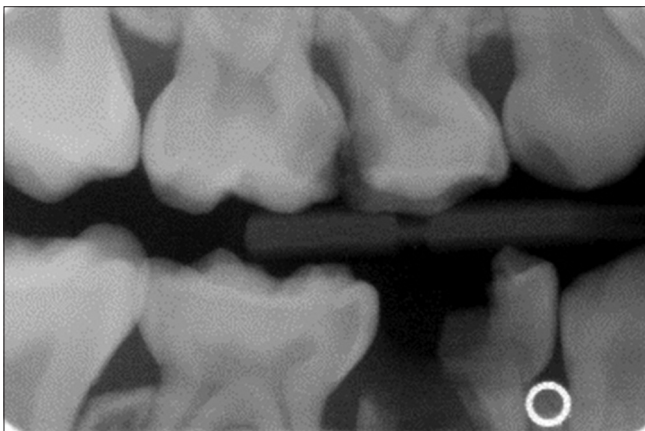
DNA was extracted using a DNA extraction system (high pure polymerase chain reaction (PCR) Template Preparation Kit; Roche, Mann-heim, Germany) according to manufacturer's recommendations. The DNA was then split into aliquots for microIdent® test. The primers for *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *P. intermedia*, *Campylobacter rectus* (Cr), and *Eikenella corrodens* were prepared as previously described by Ashimoto *et al.*<sup>[10]</sup> and those for *A. actinomycetemcomitans*, as described by Tran and Rudney.<sup>[11]</sup> In addition, *Fusobacterium nucleatum* (Fn) and *Capnocytophaga* (Ca) were tested.

Currently, participants are followed up periodically (cleaning every  $1\frac{1}{2}$  months and standardized radiographs every 3 months). At 12 months posttreatment, samples will be collected in the same manner (test and control site for each participant) and undergo DNA analysis.

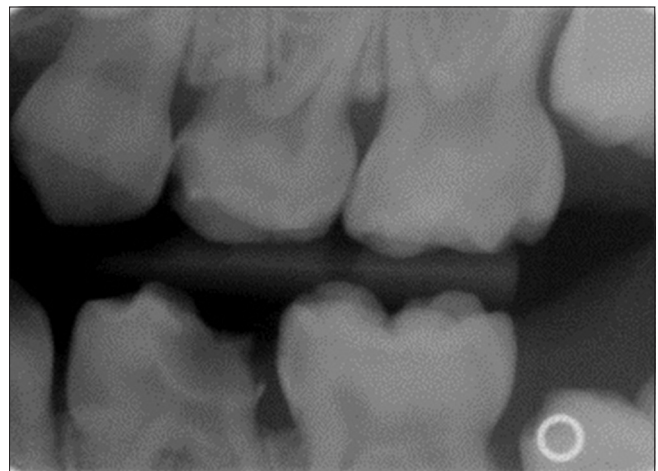
Data were analyzed using the Statistical Package for Social Sciences version 13 (SPSS, Chicago, IL, USA). Descriptive statistics were produced for all variables. Analysis of variance was used to determine any significant difference between the test and control sites. The level of significance was set at  $P \leq 0.05$ .

## RESULTS

All the 25 patients who began the study had completed all follow-up visits. In total, 300 subgingival plaque samples were analyzed.



**Figure 1:** Bitewing radiograph showing proximal caries with bone loss.



**Figure 2:** Bitewing radiograph showing proximal caries without bone loss.

The number of positive results was significantly ( $P \leq 0.01$ ) higher for Fn, Ca and Cr in children with proximal caries and bone loss (test group) compared to children having proximal caries without bone loss (control group). The lower bacterial load was found for *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *P. intermedia*, and Fn using DNA-bacterial probe technology in both the groups [Figures 3 and 4].

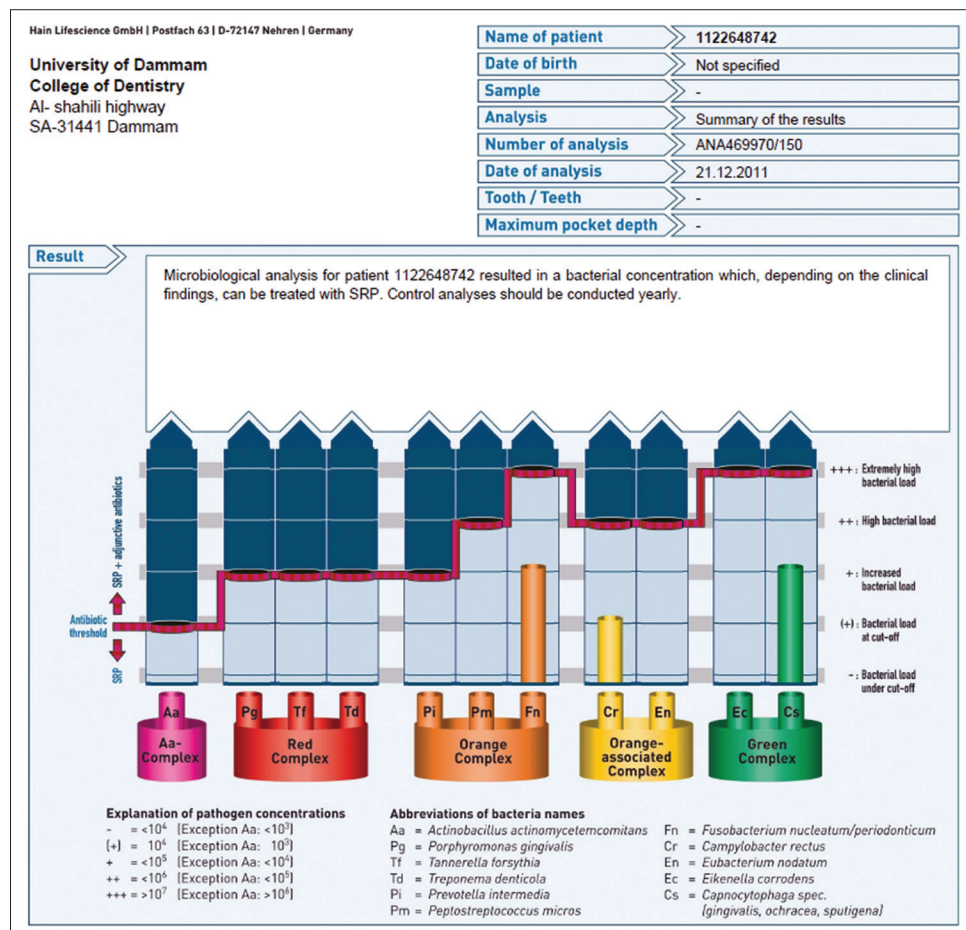
The sensitivity of microIDent® using a DNA-bacterial probe as a reference ranged between 83.5% (Ca), 76.4% (Cr), and 70% (Fn) for the major pathogens and between 40.9% (*Treponema denticola*), 40% *E. corrodens*, and 41% (*T. forsythia*) in the test group. In comparison, the sensitivity of microIDent in the control group ranged between 67.5% (Ca), 70% (Fn) and 41.4% (Cr). The lowest bacterial loads detected were 20% (*P. intermedia*), 18% (*A. actinomycetemcomitans*), and 17.4% (*P. gingivalis*) [Figures 3 and 4].

## DISCUSSION

It is widely accepted that the etiology of periodontitis is polymicrobial in nature.<sup>[12]</sup> Worsening or improvement

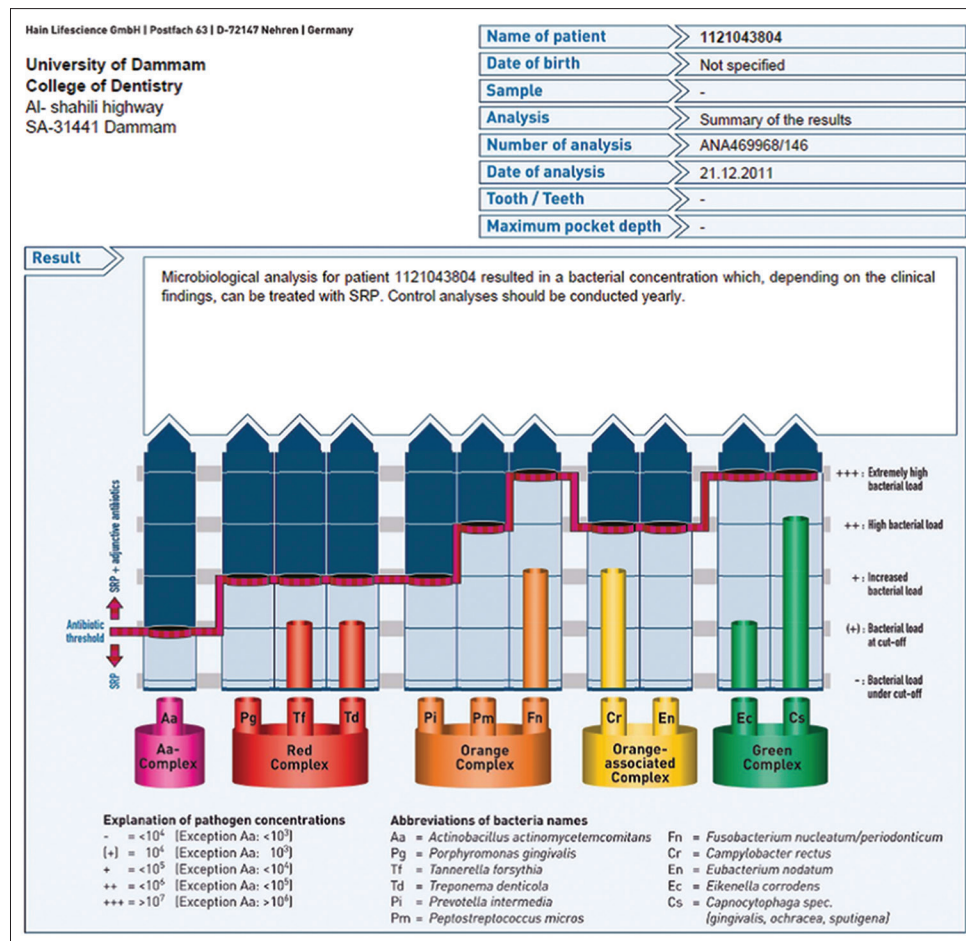
of periodontal status is accompanied by a shift in the bacterial composition of subgingival plaque.<sup>[13]</sup> It has therefore been suggested that microbial testing can be used for diagnosis and to optimize periodontal therapy and assess its outcome, especially when treatment with antimicrobial drugs is considered. However, this strategy may be confounded, since initiation and progression of periodontal disease are influenced by the interaction of myriad genetic, environmental, host, and microbial factors.<sup>[14-16]</sup>

It is necessary to carefully consider the purpose of periodontal microbiology testing in order to choose a suitable detection method.<sup>[12]</sup> Subgingival microflora consists of more than 300 different species that can be detected by different techniques. Detection methods for periodontopathogens may be divided into four general categories: Culture, immunodiagnostic, nucleic acid probe and PCR. Culturing techniques have been used for decades in the detection of periodontopathogens and are frequently used as the reference method. Most significantly, culture can provide antibiotic susceptibility



**Figure 3:** Periopathogens in children with proximal caries without bone loss





**Figure 4:** Periopathogens in children with proximal caries associated with bone loss

of *A. actinomycetemcomitans* and *P. gingivalis* and total microbial content, information that is not obtainable by other current microbial detection methods. Immunodiagnostic methods have the advantage of being fast and inexpensive. However, cross-reactivity with nontarget organisms may occur. Furthermore, immunodiagnostic methods generally provide poorer detection limit for *A. actinomycetemcomitans* and *P. gingivalis* than culture, nucleic acid probe or PCR assays. Nucleic acid probes that hybridize to species-specific regions of the genome may show a good detection limit and no cross-reactivity with other oral bacterial under optimal conditions.<sup>[17-20]</sup>

In the present study, we reevaluated the association of putative periodontal pathogens in boys of aged between 4 and 6 years having proximal caries associated with bone loss versus those without bone loss-control to identify species which are incompatible with periodontal health. We analyzed the presence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, a *T. denticola*-like phylogroup (*Treponema* phylogroup II), *Treponema lecithinolyticum*, Cr, *Fusobacterium* spp., and Fn, as

well as *Capnocytophaga ochracea*. We also examined whether the presence or absence of these species was related to alveolar bone loss. Taking these factors into consideration, although there is a potential for translocation of periodontal pathogens from one pocket to another, carefully performed plaque control measures and changes in the subgingival environment and the host response induced by treatment may prevent re-infection of sites, and thus relapse of periodontal disease.

In the present study, we did find that higher levels of *Ca sp.* in children who have proximal caries is associated with bone loss, Which confirms the findings of other researchers,<sup>[21]</sup> *Capnocytophaga sputigena* was present in higher numbers than *Capnocytophaga gingivalis* in subgingival plaque, which may be because microIDent® uses a mixed probe for Ca that might be more sensitive for *C. gingivalis*.

Also, in this study, we found a high bacterial load of Fn and Cr. Our results are agreement with several studies, which reported that Cr, *Eubacterium nodatum*,

*E. corrodens* as well as *Fn*, *P. intermedia*, and *Parvimonas micra* are known to be associated with clinical periodontal disease.<sup>[22-25]</sup>

## CONCLUSION

In the study confirmed that there was a relationship between microbial pathogens associated with proximal dental caries in the primary dentition and periodontal disease in children. In addition, DNA microbial probe testing technology clearly analyzed the different loads of periodontopathogens in children who had proximal caries associated with bone loss and is useful in microbial diagnostics for patients in dental practices.

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## Conflicts of interest

There are no conflicts of interest.

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